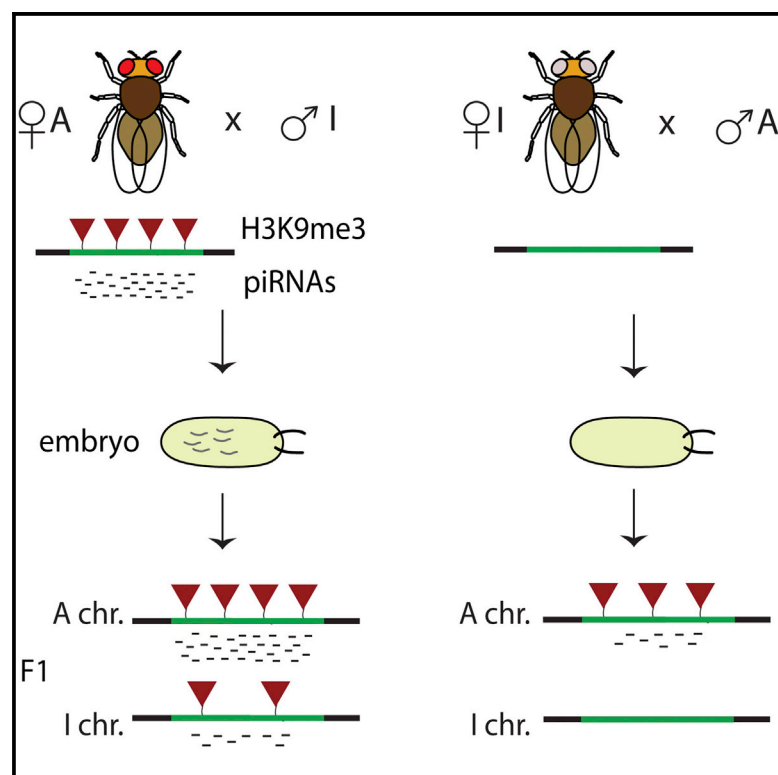


A Transgenerational Process Defines piRNA Biogenesis in *Drosophila virilis*

Graphical Abstract



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In Brief

Small noncoding RNA molecules called piRNAs, encoded by specific genomic regions, silence the expression of selfish transposable elements in *Drosophila* germ cells. What defines a genomic region for piRNA production is unknown. In this study, Le Thomas et al. show that active piRNA biogenesis requires high levels of H3K9me3 that cover the piRNA-generating regions. Furthermore, piRNA generation in the progeny requires maternal deposition of an epigenetic signal, presumably a piRNA, that establishes the H3K9me3 mark.

Highlights

piRNA cluster activity in *D. virilis* correlates with high levels of H3K9me3

A transgenerational signal induces piRNA biogenesis in progeny

An inherited maternal signal causes H3K9me3 deposition at piRNA-producing regions

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A Transgenerational Process Defines piRNA Biogenesis in *Drosophila virilis*

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SUMMARY

Piwi-interacting (pi)RNAs repress diverse transposable elements in germ cells of Metazoa and are essential for fertility in both invertebrates and vertebrates. The precursors of piRNAs are transcribed from distinct genomic regions, the so-called piRNA clusters; however, how piRNA clusters are differentiated from the rest of the genome is not known. To address this question, we studied piRNA biogenesis in two *D. virilis* strains that show differential ability to generate piRNAs from several genomic regions. We found that active piRNA biogenesis correlates with high levels of histone 3 lysine 9 trimethylation (H3K9me3) over genomic regions that give rise to piRNAs. Furthermore, piRNA biogenesis in the progeny requires the transgenerational inheritance of an epigenetic signal, presumably in the form of homologous piRNAs that are generated in the maternal germline and deposited into the oocyte. The inherited piRNAs enhance piRNA biogenesis through the installment of H3K9me3 on piRNA clusters.

INTRODUCTION

A distinct class of small RNAs called Piwi-interacting RNAs, or piRNAs, provides sequence specificity for the recognition and repression of a diverse set of invasive genetic elements in the germline of metazoans (Aravin et al., 2007; Siomi et al., 2011). piRNAs are loaded into members of the Piwi clade of Argonaute proteins. Piwi/piRNA complexes in *Drosophila* repress transposable elements through two different mechanisms. In the nucleus, piRNAs associated with PIWI are responsible for the deposition of H3K9me3 on homologous transposon targets (Le Thomas et al., 2013; Rozhkov et al., 2013; Sienski et al., 2012). In the cytoplasm, two other Piwi proteins, AUB and AGO3, are guided by the associated piRNAs to cleave and destroy homologous transposon transcripts using their endonuclease activity. Simultaneously, their cleavage of complementary transcripts by AUB and AGO3 also leads to the biogenesis of new piRNAs (Brennecke et al., 2007).

The proper selection of RNA molecules to be processed into mature piRNAs is critical for generating the diverse repertoire

of piRNAs that guide effective silencing of transposable elements. The genomic regions that give rise to piRNAs, the piRNA clusters, define the repertoire of elements that are recognized and silenced. However, how piRNA clusters are differentiated from the rest of the genome is not known. piRNAs are distinct from other classes of small RNAs, such as microRNA (miRNA) and small interfering RNA (siRNA), which are channeled into their respective biogenesis pathways by the distinct secondary structure of their precursors (Kim et al., 2009). In contrast, no structural or sequence motifs have been identified in piRNA precursors in *Drosophila*. In fact, any sequence inserted into piRNA clusters is processed into mature piRNAs (Muerdter et al., 2012; Todeschini et al., 2010). In a recent breakthrough study, de Vanssay and colleagues showed that piRNAs derived from a transgenic locus are able to induce de novo piRNA biogenesis at a homologous locus originally incapable to generate piRNAs (de Vanssay et al., 2012), yet the mechanisms behind this phenomenon remained unclear.

Several studies have suggested that piRNA clusters in *Drosophila* possess a distinct chromatin structure that is important for piRNA biogenesis. Indeed, piRNA clusters expressed in *D. melanogaster* germ cells are enriched for H3K9me3 (Rangan et al., 2011). They are also enriched for heterochromatin protein 1 (HP1) (Moshkovich and Lei, 2010) and its germline specific paralog, Rhino (Klattenhoff et al., 2009), which have chromodomains capable of binding to H3K9me3 (Le Thomas et al., 2014). Furthermore, deficiency in the histone methyltransferase Eggless/SETDB1, which is responsible for deposition of the H3K9me3 mark, and Rhino leads to the loss of piRNAs in germ cells (Rangan et al., 2011; Klattenhoff et al., 2009).

Here, we built upon our previous finding of two *Drosophila virilis* strains that show differential capacity to generate piRNAs from several genomic loci (Rozhkov et al., 2010) in order to understand the genetic and epigenetic requirements for piRNA biogenesis. We found that piRNA biogenesis positively correlates with the level of H3K9me3 on genomic loci. Furthermore, maternally inherited epigenetic factors, likely piRNAs, are necessary for the maintenance of high H3K9me3 levels on homologous loci in the progeny.

RESULTS

To understand the determinants of piRNA cluster activity and identify the features that discriminate piRNA-generating regions

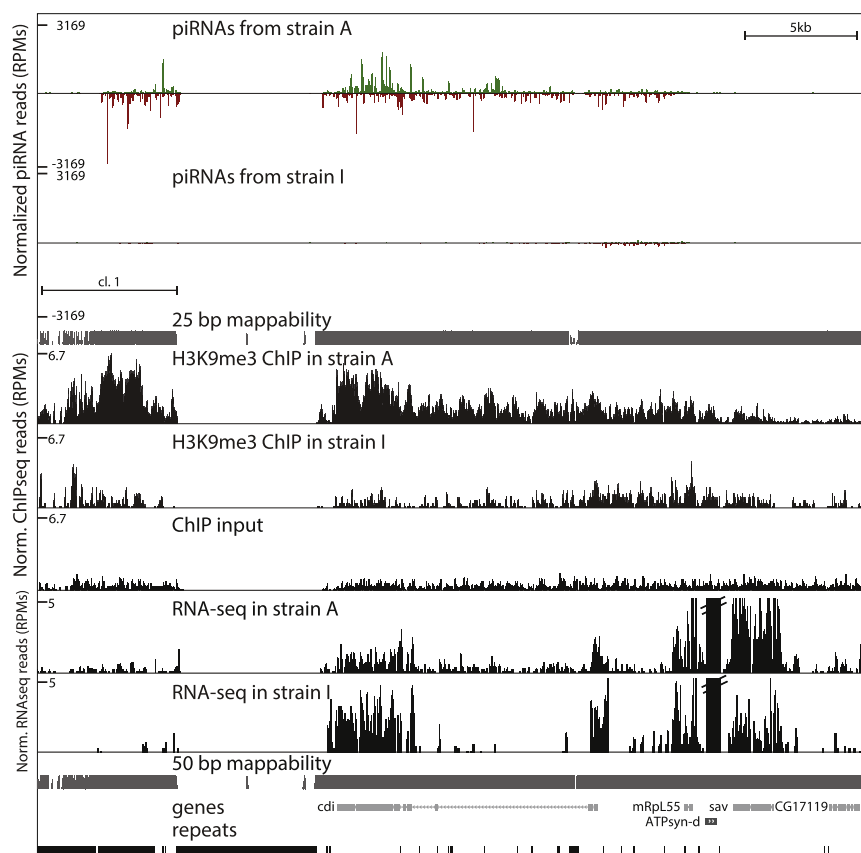


Figure 1. Profiles of the H3K9me3 Mark, piRNAs, and Long RNAs on Differentially Expressed piRNA Clusters

piRNA, H3K9me3, and long RNA-seq (rRNA-depleted total RNA) densities on cluster #1 in the two *D. virilis* strains. High H3K9me3 signal is present over the cluster sequence in strain A that generates abundant piRNAs from this region; low H3K9me3 signal and no piRNAs are present in strain I. Only uniquely mappable reads are shown, resulting in the gap in read density over a recent and nondivergent repetitive element (LTR retrotransposon) insertion. Unique-mappability tracks for 25- and 50-bp-long reads are shown in gray. piRNAs mapping to the forward (“+”) and reverse (“−”) strand are shown in green and red, respectively. See also Table S4.

this strain compared to strain I (Figures 1, 2A, 2B, and S1A). In contrast, cluster #4, which produced a similar amount of piRNAs in both strains, had comparable levels of H3K9me3 (Figures 2A, 2B, and S1A). The H3K9me3 profile on piRNA clusters in strain A closely parallels the profile of piRNA generation: both signals drop at the same genomic position (Figure 1). Therefore, the activity of native piRNA-generating regions in *D. virilis* correlates with high levels of the H3K9me3 mark for all three differentially expressed clusters.

from other genomic loci, we took advantage of our previous finding of piRNA clusters that are differentially active in two *D. virilis* strains (Rozhkov et al., 2010). Three genomic regions designated as clusters #1, #2, and #3 are present in the genomes of both strains 9 and 160, yet piRNAs are only generated from these clusters in strain 160 (we will refer to strain 160 as the active or A strain) (Figures 1 and 2A). Strain 9 (the inactive or I strain) does not have general defects in the piRNA pathway as piRNAs are generated from other genomic regions in this strain. The reason for the differential ability to generate piRNAs from certain genomic regions in the two strains is unknown.

Recent studies revealed that two chromatin factors, SETDB1, a methyltransferase responsible for installation of H3K9me3, and the HP1 paralog Rhino that binds this mark are required for piRNA biogenesis in *D. melanogaster* (Klattenhoff et al., 2009; Rangan et al., 2011). We hypothesized that a difference in the chromatin state of the genomic regions in strains A and I might explain their differential ability to produce piRNAs. To determine whether chromatin state plays a role in the differential activity of the piRNA clusters, we profiled H3K9me3 in ovaries from flies of both strains. For each strain, chromatin immunoprecipitation sequencing (ChIP-seq) libraries from two independent biological samples were generated, sequenced, and analyzed. Genomic regions that generate piRNAs exclusively in strain A (clusters #1–3) had significantly higher levels of H3K9me3 in

To understand how H3K9me3 affects the activity of piRNA-generating loci, we used ChIP-seq to profile genome-wide RNA polymerase II occupancy and RNA-seq to measure transcript levels in ovaries from strains A and I. We found that high H3K9me3 levels on differentially expressed clusters in strain A correlated with high occupancy of RNA polymerase II on these regions (Figures 2C and S1B). Furthermore, the piRNA clusters gave rise to more precursor transcripts in strain A than in strain I, indicating that the enrichment of Pol II at these sites represents polymerase engaged in active transcription (Figures 2D and S2). Together, these results suggest that high levels of H3K9me3 on piRNA clusters correlate with the generation of precursor transcripts for piRNA processing.

To further understand the properties of genomic regions that generate piRNAs, we studied the activity of piRNA clusters in the progeny of the cross between strains A and I. The cross between the two strains can be performed in two different directions: the progeny of the cross between strain A females and strain I males was designated as MD (for maternal deposition) as the A chromosomes and corresponding piRNAs are inherited from the mothers (Figure 3A). In the progeny of the opposite cross, designated as NMD (for no maternal deposition), the A chromosomes are inherited from the fathers, and no cognate piRNAs are inherited. Importantly, the genotypes of MD and NMD progeny are absolutely identical.

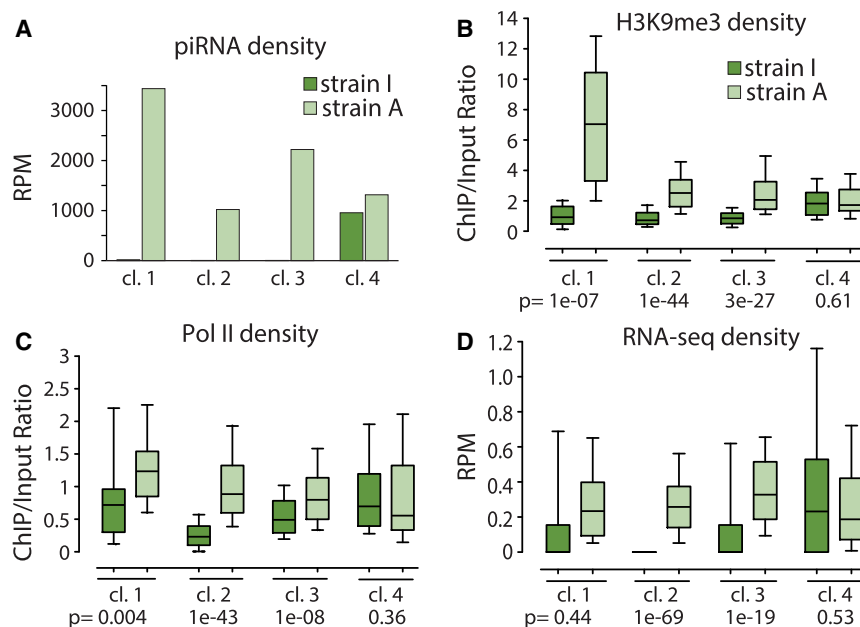


Figure 2. Enrichment of H3K9me3 on piRNA Clusters Correlates with Increased Transcription and piRNA Generation

(A) Differential expression of piRNA clusters in the two *D. virilis* strains. Clusters #1, #2, and #3 generate large numbers of piRNAs in strain A but are inactive in strain I. Cluster #4 shows similar piRNA levels in both strains. Only the 5' portion of cluster 1, which does not contain protein-coding sequences (marked on Figure 1), was used for quantification (here and in subsequent analyses).

(B) Production of piRNAs correlates with H3K9me3 levels. Clusters #1, #2, and #3 have high levels of H3K9me3 in strain A and low levels in strain I; cluster #4 has similar levels of H3K9me3 in both strains as measured by ChIP-seq. An independent biological replicate for this experiment is shown in Figure S1A.

(C) RNA polymerase II density over piRNA clusters in the two strains as measured by ChIP-seq. Clusters #1, #2, and #3 have higher RNA Pol II density in strain A, whereas cluster #4 shows similar levels of RNA Pol II signal in both strains. Because cluster #1 contains a protein-coding gene, only sequences coming from the regions not

overlapping with the gene were considered. An independent biological replicate for this experiment is shown in Figure S1B.

(D) Long RNA expression from piRNA clusters in the two strains as measured by RNA-seq on rRNA-depleted total RNA. Clusters #1, #2, and #3 generate more transcripts in strain A than in strain I; levels were similar in the two strains for cluster #4. For (B)–(D), cluster genomic regions were divided into 200 bp windows, a sequencing-depth normalized RPM (reads per million) score was calculated for each window, and the distribution of values was plotted. The Mann-Whitney U test was used to calculate the p values and evaluate the significance of the observed differences.

See also Figures S1 and S2 and Table S4.

Profiling of piRNAs in ovaries of MD and NMD flies showed that similar amounts of piRNAs were generated from cluster #4, which is equally expressed in both parental strains (Figure 3B). Surprisingly, ~3-fold more piRNAs were generated from the three differentially active clusters in MD compared to NMD progeny. Because the genotypes of MD and NMD progeny are identical, this result indicates that the transgenerational inheritance of an epigenetic signal that is transmitted from the mothers to their progeny enhances the ability to generate piRNAs.

One mechanism of piRNA biogenesis involves the ping-pong amplification loop. In the ping-pong loop, piRNAs guide cleavage of complementary targets leading to the generation of new piRNAs from the cleaved product (Brennecke et al., 2007; Gunawardane et al., 2007; Olovnikov and Kalmykova, 2013). These secondary piRNAs belong to complementary piRNA pairs with a 10 nt overlap between their 5' ends (so called ping-pong pairs). In the MD progeny, the majority of piRNA sequences coming from clusters 1–3 can form pairs with a 10 nt overlap between their 5' ends (Figure 3C). In contrast, only a small fraction of piRNAs form ping-pong pairs in the NMD progeny. It must be noted, however, that, because of limited sequencing depth, this fraction underestimates the real proportion of secondary piRNAs, especially for the piRNA pairs that produce few reads of one partner. In an attempt to account for the different number of sequences derived from differentially expressed clusters in MD and NMD progenies, we sampled the same number of reads from each library and calculated the fraction of reads in ping-pong pairs a thousand times. A similar proportion of secondary

piRNAs was detected in strain A and in the MD progeny; however, the fraction of piRNAs forming ping-pong pairs in clusters #1, #2, and #3 was dramatically reduced in the NMD progeny, compared to the fraction in the MD progeny (Figure 3D). A similar fraction of piRNAs mapping to cluster #4 formed ping-pong pairs in strain A and both the MD and the NMD progeny, indicating that the ping-pong machinery was intact in both progenies. These data suggest that an epigenetic signal inherited by MD progeny from their mothers eventually boosts the biogenesis of secondary piRNAs.

Next, we studied the chromatin state of differentially active clusters in MD and NMD progenies. We compared the levels of H3K9me3 using separate ChIP-qPCR and ChIP-seq experiments performed on two independent biological samples of MD and NMD ovaries (Figures 4A and S3). In both progenies, H3K9me3 levels over the clusters were intermediate between the strong and weak enrichment seen in strain A and strain I, respectively. Importantly, both methods show that H3K9me3 enrichment was higher in ovaries of MD compared to NMD flies, indicating that the maternally supplied epigenetic signal affects chromatin of piRNA clusters in the progeny (Figures 4A and S3).

The identical genomes of the MD and the NMD progenies each contain two alleles of differentially active piRNA clusters that have different H3K9me3 levels in their parents, the A and the I strains. To understand the impact of the maternally supplied epigenetic signal on individual alleles, it is critical to differentiate sequences derived from the A and I chromosomes. We therefore carried out whole-genome sequencing of the genomes of both

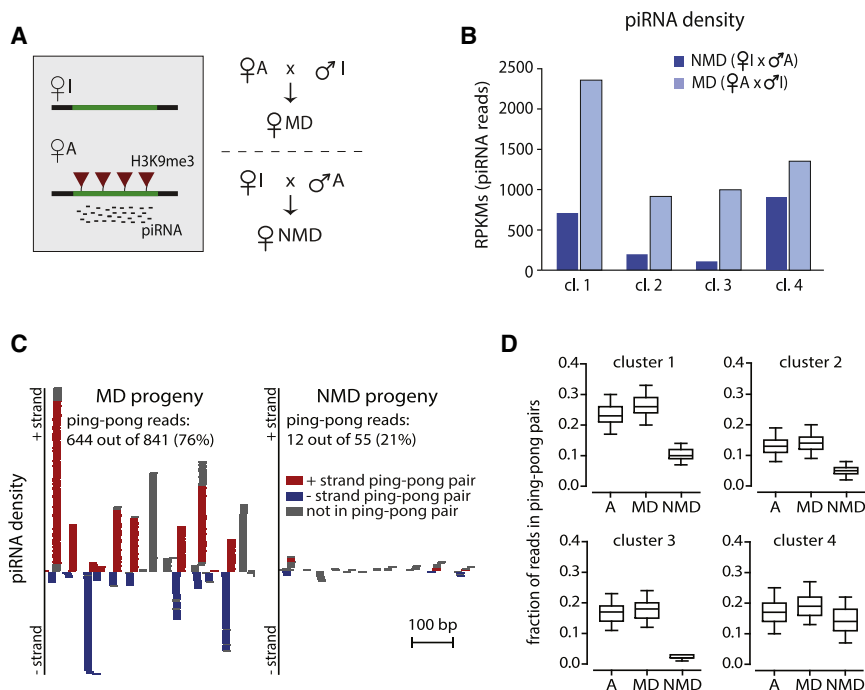


Figure 3. The Effect of Transgenerational Epigenetic Signal on the Ping-Pong piRNA Processing

(A) Scheme of the cross between A and I strains along with the chromatin status and piRNA levels at differentially expressed clusters in the parental strains. H3K9me3 levels (red triangle) and piRNA abundance for the parental strains are shown in the gray box.

(B) Transgenerational inheritance of piRNAs from the mother leads to increased piRNA generation in the progeny. Expression of piRNAs in MD progeny (female A crossed to male I) that inherited piRNAs derived from clusters #1, #2, and #3 from their mothers and NMD progeny (female I crossed to male A) that did not inherit these piRNAs.

(C) The fraction of piRNAs in ping-pong pairs is higher in MD progeny than in NMD progeny. piRNA density on a 500 bp fragment of cluster #1 shows higher numbers of ping-pong pairs in the MD progeny than in the NMD progeny. Shown are individual piRNA reads with reads in ping-pong pairs on the forward and reverse strand in red and blue, respectively.

(D) Transgenerational epigenetic signal enhances the ping-pong amplification cycle in the progeny as indicated by the higher abundance of ping-pong pairs in MD compared to NMD progeny. To

determine the fraction of piRNAs that participate in ping-pong pairs uniquely mapped, piRNA reads that mapped to opposite strands of each other and have a 10 bp distance between their 5' ends were counted. To account for variable sequencing depth and for the different number of reads over each cluster in different samples, we carried out the analysis by sampling reads to ~25% of the read counts in the library with the fewest reads in a region and repeating this 1,000 times (see [Experimental Procedures](#) for details).

See also [Table S4](#).

strains. We generated 82 times and 71 times coverage for strains A and I, respectively, and identified SNPs for each strain relative to the *D. virilis* reference genome (see the [Experimental Procedures](#) for details). We found 326,026 and 1,086,963 SNPs in the genomes of A and I, respectively, of which, 169,192 SNPs were shared. A total of 1,074,605 SNPs differed between the genomes of the two strains (average density of 5.19 such SNPs per Kb) allowing us to determine the allelic origin of piRNAs and ChIP-seq reads derived from polymorphic genomic regions. Importantly, we identified such SNPs in each of the differentially expressed piRNA clusters ([Table S1](#)). This allowed us to unambiguously map a fraction of the ChIP-seq and piRNA reads to each individual allele ([Figures 4B–4D](#) and [S4](#)).

We found that the two alleles of differentially expressed piRNA clusters maintained their distinct chromatin states in the hybrid progeny. Levels of H3K9me3 were higher on the A alleles compared to the I alleles in both the MD and the NMD ovaries ([Figures 4B](#) and [S4](#)). However, MD progenies had higher levels of H3K9me3 mark on both the A and I alleles. This result indicates that maternally inherited epigenetic signal in MD flies causes an increase in H3K9 methylation on the I alleles. Importantly, the increase in H3K9me3 signal on the I alleles in the MD progeny correlated with the induction of piRNA generation from these alleles, whereas no piRNAs were generated from the I alleles in the NMD flies ([Figures 4C](#) and [4D](#)). Therefore, transgenerational inheritance of an epigenetic signal correlates with deposition of the H3K9me3 mark and induction of piRNA generation from previously inactive genomic loci ([Figure 4E](#)).

DISCUSSION

Our results reveal an essential role for a transgenerationally inherited epigenetic factor in generation of piRNAs in germ cells of *D. virilis*. This maternally inherited factor is required to maintain the high level of piRNA generation from active piRNA clusters. Furthermore, it is able to activate previously naive loci on I chromosomes. These findings parallel the study by de Vanssay and colleagues who showed that a maternally inherited factor produced by a transgenic piRNA locus is able to activate piRNA generation from a previously inactive homologous locus in *D. melanogaster* (de Vanssay et al., 2012). Multiple lines of evidence point to piRNAs themselves as the carriers of the epigenetic signal that triggers piRNA generation from homologous loci in the progeny. First, in *Drosophila* both piRNAs and Piwi proteins are inherited from the maternal germline to the early embryos (Brennecke et al., 2008). Second, piRNAs can serve as sequence-specific guides to identify and activate homologous loci. Recent studies have shown that piRNAs and the nuclear Piwi protein trigger installation of the H3K9me3 mark on homologous targets providing a possible mechanism by which inherited piRNAs could lead to chromatin changes (Le Thomas et al., 2013; Rozhkov et al., 2013; Sienski et al., 2012; Wang and Elgin, 2011). Finally, the study of a similar phenomenon in *D. melanogaster* showed that the epigenetic signal produced by the transgenic piRNA locus does not require inheritance of the locus itself (de Vanssay et al., 2012). This result indicates that the epigenetic signal has a nonchromosomal nature and

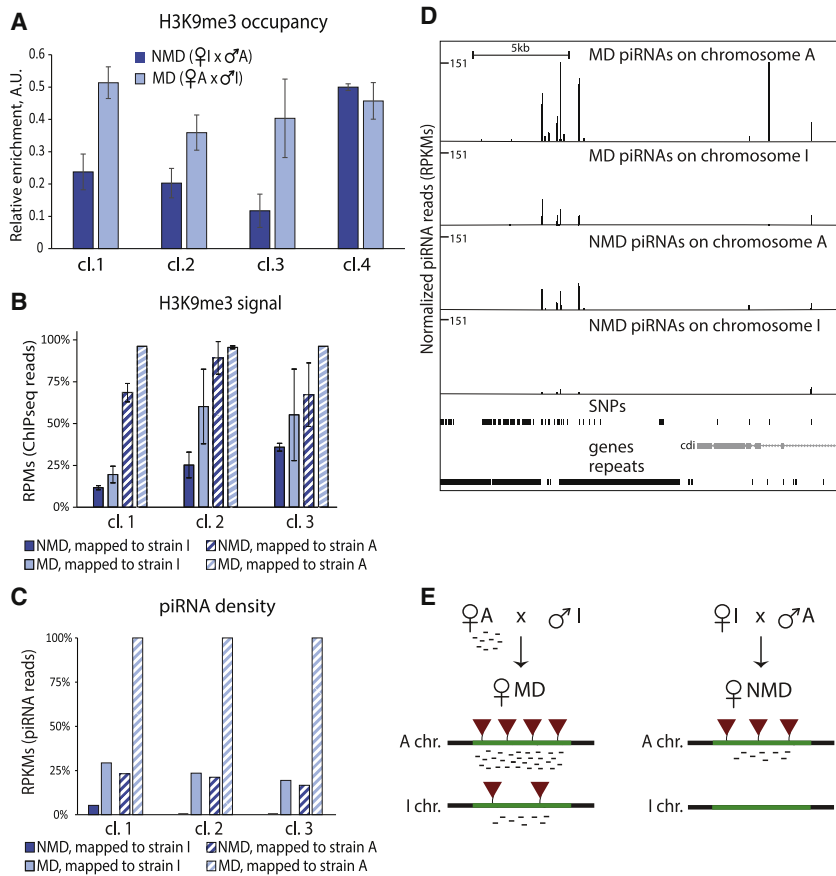


Figure 4. The Effect of Transgenerational Epigenetic Signal on the Activity and the Chromatin State of piRNA Clusters

(A) Transgenerational epigenetic signal leads to increase in H3K9me3 levels on piRNA clusters in the progeny. The input-normalized H3K9me3 signal as measured by ChIP-qPCR is higher in the MD progeny compared to the NMD progeny. ChIP-qPCR signals were normalized to respective inputs and compared to signal at a control genomic region located inside cluster #4 (scaffold_12823:176535-176654). The levels of H3K9me3 on the same clusters measured by ChIP-seq performed on two independent biological samples are shown in Figure S3. The error bars represent the SEM between two independent biological replicates.

(B) Maternally inherited piRNAs lead to an increase in H3K9me3 levels on both the paternal and maternal alleles in the progeny. H3K9me3 ChIP-seq data were mapped to the heterozygous genome of the cross between A and I, considering only reads that could be unambiguously mapped to either the I or the A genome (see Experimental Procedures for details). For each cluster, the H3K9me3 levels are normalized to the signal on allele A in the MD cross. For both the I and the A chromosomes, H3K9me3 levels over the differentially expressed clusters increase when cognate piRNAs are inherited (MD versus NMD progeny). At the same time, each parental chromosome preserves its distinct chromatin state in the progeny: H3K9me3 levels are higher on the parental chromosome coming from strain A than on the one coming from strain I. The error bars represent the SD of the mean between two independent biological replicates. See also Figure S4.

(C) Inheritance of piRNAs boosts piRNA production from both parental chromosomes in the progeny. piRNA density on each chromosome was determined by mapping reads to the heterozygous genome of the cross between A and I, considering only reads that could be unambiguously mapped to either the I or the A genome. For each cluster, the number of piRNAs is normalized to the piRNA derived from allele A in MD cross. For each differentially expressed cluster, more piRNAs are produced from each allele when piRNAs are maternally inherited (MD versus NMD progeny). Importantly, inheritance of piRNAs activates piRNA generation from clusters that were inactive in the parental I strain (solid light blue versus dark blue bars).

(D) The profiles of piRNA derived from A and I chromosomes in MD and NMD progenies on cluster #1. Only reads that mapped to SNPs that are distinct between the genomes of strain A and I are shown. Note that chromosome I generates piRNAs in MD, but not NMD flies. The distribution of SNPs is shown below piRNA tracks. See also Figure S4.

(E) Scheme of the cross between A and I strains along with the chromatin status (the level of H3K9me3 mark, red triangles) and the piRNA levels of each chromosome in the progeny.

See also Figures S3 and S4 and Tables S1, S3, and S4.

eliminates the possibility that it is carried by a chromatin mark linked to the active locus. Our results, together with these previous studies, strongly support the role of inherited piRNAs as a transgenerationally inherited epigenetic signal that activates piRNA generation from homologous loci in the progeny.

Transgenerationally inherited piRNAs activate piRNA generation in a nuclear process that is linked to the deposition of H3K9me3 on homologous genomic regions. We found that acquisition of the H3K9me3 mark by genomic regions that did not previously produce piRNAs correlates with initiation of piRNA biogenesis (Figure 4). In contrast, absence of inherited piRNAs leads to a decrease in H3K9me3 levels on homologous regions and a concomitant decrease in the corresponding piRNAs (Figure 4). These results suggest that modification of the chromatin structure of genomic regions homologous to inherited piRNAs induces piRNA biogenesis in the progeny. In

agreement with our results, previous studies have shown that the biogenesis of piRNAs in *D. melanogaster* requires the activity of Eggless/SETDB1, one of the methyl-transferases carrying out the trimethylation of H3K9 (Rangan et al., 2011) and of Rhino, the HP1 homolog, that is enriched over piRNA clusters (Klattenhoff et al., 2009; Zhang et al., 2014). Rhino has a chromodomain that is similar to the chromodomain of HP1 and binds the H3K9me3 mark (Le Thomas et al., 2014; Mohn et al., 2014).

The exact molecular mechanism by which the H3K9me3 mark is linked to piRNA biogenesis in the nucleus remains to be elucidated; however, our results suggest that, counterintuitively, high levels of H3K9me3 correlate with elevated transcription in the context of piRNA clusters. Indeed, we found that high levels of H3K9me3 on differentially expressed piRNA clusters in *D. virilis* correlated with increased Pol II occupancy and with the generation of more precursor transcripts (Figures 1 and 2). Therefore,

we propose that changes in chromatin state associated with high levels of H3K9me3 lead to the recruitment of nonconventional readers of this mark such as Rhino, which subsequently affect transcription in these regions, providing more precursors for piRNA biogenesis. Overall, our data revealed that the determination of a piRNA producing loci in *D. virilis* is mediated by the process of the transgenerational epigenetic inheritance.

EXPERIMENTAL PROCEDURES

Drosophila Stocks

D. virilis strain 160 (A) and strain 9 (I) were a generous gift from M. Evgeyev.

ChIP and RNA-Seq

ChIP experiments were carried out using commercially available antibodies anti-H3K9me3 (ab8898) and anti-RNA Pol II (ab5408). Ovaries were fixed for 10 min at room temperature using 1% paraformaldehyde (PFA) followed by 5 min quenching by directly adding glycine (final concentration 25 mM). A detailed protocol is provided in the [Supplemental Experimental Procedures](#). Quantitative ChIP-PCR (ChIP-qPCR) experiments were performed in at least two biological replicates with two technical replicates each. Error bars represent the SEM. Primer pairs used in the qPCR experiments are presented in [Table S3](#). ChIP-seq and RNA-seq library construction and sequencing were carried out using standard protocols and sequenced on the Illumina HiSeq 2000 (50 bp reads). Publicly available data sets for piRNAs were extracted from the GEO Short Read Archive GSE22067 ([Rozhkov et al., 2010](#)).

Analysis of Ping-Pong Processing

To determine the fraction of piRNAs that participate in ping-pong pairs, we counted uniquely mapped piRNA reads that map to opposite strands of each other, overlap, and have a 10 bp distance between their 5' ends. To account for different number of piRNAs in each sample, we carried out the analysis by sampling reads to ~25% of the read counts in the library with the fewest reads in a region and repeating this 1,000 times.

D. virilis Genome Sequencing and Allele-Specific Mapping

Genomic libraries were generated from each *D. virilis* strain and sequenced (as 2 × 100 reads) on an Illumina HiSeq 2000 platform. Sequencing coverage was ~82 times for strain A and ~71 times for strain I. A detailed protocol for allele-specific mapping is provided in the [Supplemental Experimental Procedures](#). Detailed statistics on the number of SNPs identified genome-wide and in the particular piRNA clusters studied here can be found in [Table S1](#).

ACCESSION NUMBERS

High-throughput sequencing data for ChIP-seq and RNA-seq experiments are available through the Gene Expression Omnibus (accession number GSE59965).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and four tables and can be found with this article online at <http://dx.doi.org/10.1016/j.celrep.2014.08.013>.

AUTHOR CONTRIBUTIONS

A.L.T. and A.A.A. designed the experiments, A.L.T. performed the experiments, G.K.M. analyzed the deep sequencing data, and A.L.T., G.K.M., and A.A.A. interpreted the data and wrote the manuscript.

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